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Frost tolerance of lumbricid earthworm cocoons

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With 2 figures

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1. Introduction

The upper soil layer in the morthern hemisphere is often frozen for shorter or longer periods during winter, so organisms living there could benefit from an ability to tolerate frost to some degree.

Previous investigation have shown that earthworms are very vulnerable to temperatures below 0 °C (HOPP, 1947; HOPP & LINDER, 1947). Usually they attempt to avoid such low temperatures by migrating vertically into deeper and warmer soil layers during cold periods (RUNDGREN, 1975). However, situations may occur where worms are prevented from migrating, e.g. if the temperature drops quickly or where the soil layer is shallow.

Field observations from Finland (HUHTA, 1978), for example, showed that a period with hard frost in the soil killed almost all postembryonic worms, so that in the following spring only small juveniles newly hatched from cocoons were found in samples. This indicates that the earthworm cocoons could withstand frost better than the postembryonic individuals. However, the degree of frost tolerance of cocoons remains to be investigated.

This paper examines to what degree cocoons can survive frost and whether there are differences in frost tolerance between species.

2. Materials and methods

Earthworm cocoons obtained from laboratory cultures were used in this study.

The species represented were *Aporrectodea caliginosa* (SAV.), *A. longa* (UDE), *A. rosea* (SAV.), *Allolobophora chlorotica* (SAV.), *Lumbricus terrestris* (L.), *L. festivus* (SAV.), *Dendrodrilus rubidus* (SAV.), *Dendrobaena octaedra* (SAV.) and *Eisenia fetida* (SAV.).

Earthworms were held in 1-litre plastic pots with lids, small holes in the lids allowing ventilation. Each species was held seperately with 3–10 individuals per pot, depending on the species. All pots were kept in darkness and at a constant temperature of 15 °C.

The culture substrate was a loamy soil with a moisture content of 16–18 % (based on hygromass) and the earthworms were fed on cowdung mixed with soil in equal parts per volume. Prior to use, the soil in the cowdung mixture was wet sieved through a 2 mm mesh to hinder foreign cocoons being inadvertently introduced to the culture. Also before use, the cowdung and soil used as culture substrate was dried at 80 °C in order to kill any worms, cocoons or predators present.

Cocoons were obtained from the cultures at intervals of 3 weeks by wet sieving through a 1 mm sieve. The cocoons of each species, up to 3 weeks old were placed in petri dishes on wet filter paper and acclimatized at 5 °C for 2 d.

From 7–10 cocoons were placed in moist soil (16 % moisture, based on hygromass) in test tubes (diam. 20 mm; height 180 mm) and care was taken that cocoons did not touch one another or the wall of the test tube. The test tubes were gently tapped to ensure that the cocoons were in close contact with the soil.

The test tubes were then exposed to frost by placing them in a Hetofrig cooling bath (type CB 4) containing anti-freeze in which the temperature could be precisely (±0.1 °C) regulated. Three different experiments were carried

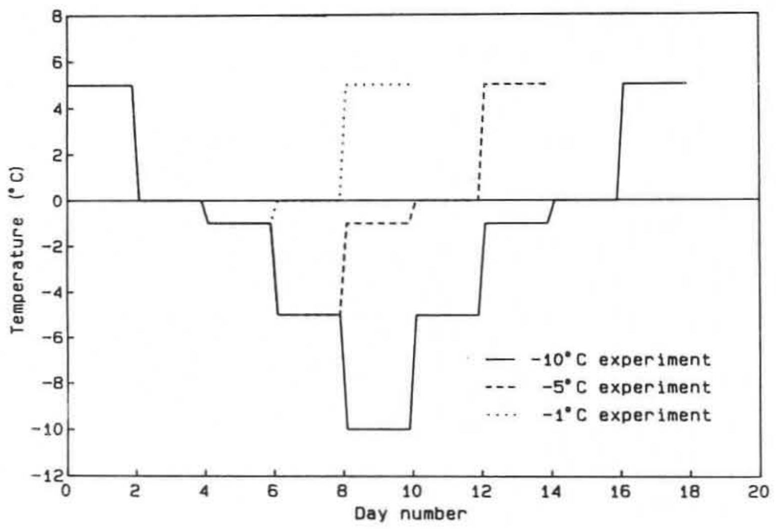

Fig. 1. Change in temperature with time for the three experiments.

out with cocoons being exposed to minimum temperature of $-1°$, $-5°$, and -10 °C, respectively. To simulate a "natural" winter situation, the temperature was regulated gradually both at decreasing and increasing temperatures. Temperature change with time in the 3 experiments is shown in fig. 1.

At the end of each experiment the cocoons were washed out of the test tubes, placed in petri dishes on wet filter paper and maintained at 20 °C. The lids of the petri dishes had small holes for ventilation. Hatching was recorded twice a week and cocoons were considered dead if there was no sign of a developing embryo after 4 weeks more than the normal incubation time at 20 °C. Incubation time at 20 °C for each species was established in a previous study (table 1).

Table 1. Normal incubation time at 20 °C for cocoons of the species tested (unpublished study).

Species	Incubation time (days)		Number of cocoons examined
	mean	S.D.	
A. caliginosa	40	5	489
A. rosea	43	8	66
A. chlorotica	39	5	222
A. longa	53	10	83
L. terrestris	58	6	142
L. festivus	53	5	86
E. fetida	37	7	699
D. rubidus	32	5	378
D. octaedra	52	8	219

In each experiment, for each species 3 test tubes were exposed to frost and one was used as a control. The control test tubes were placed in antifreeze and maintained at 5 °C while the freezing treatments were in progress.

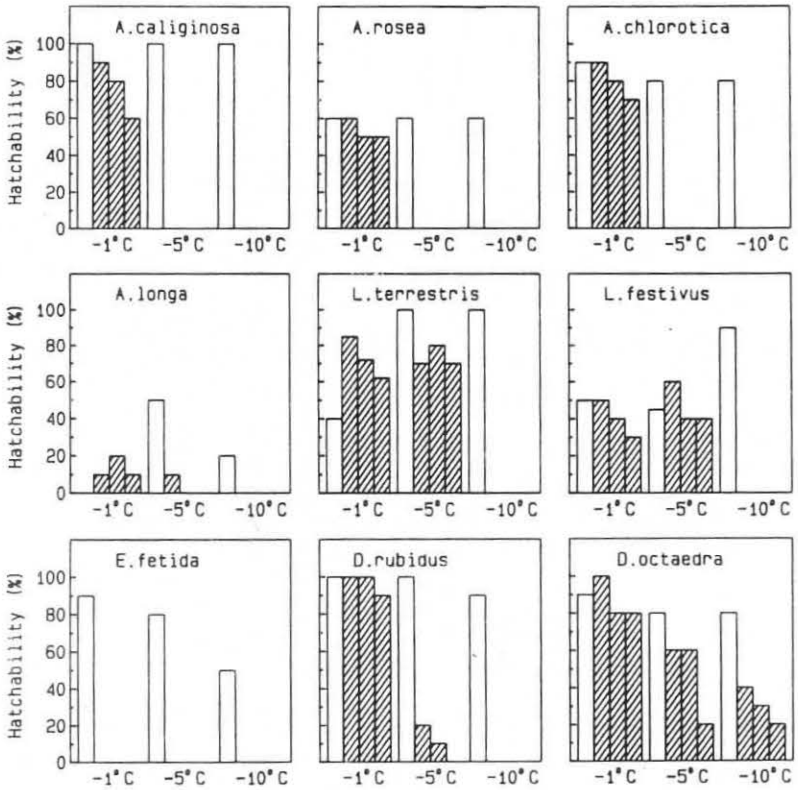

Fig. 2. Hatchability of the cocoons exposed to frost and control cocoons. Diagonally hatched columns represent test tubes exposed to frost, and white columns represent control test tubes.

3. Results

Fig. 2 shows the percent hatchability of the cocoons exposed to frost and the controls.

E. fetida cocoons did not survive frost exposure in any of the experiments whereas approximately 75% of the control cocoons hatched.

After exposure to −1 °C the hatchability of *A. caliginosa* was significantly lower ($p < 0.05$; chi squared test) than in the control. For all other species there was no significant effect of this temperature on hatching.

A. caliginosa, *A. rosea* and *A. chlorotica* cocoons did not hatch after exposure to −5 °C or −10 °C.

A. longa cocoons had a very low hatchability even in the controls. It is therefore difficult to draw any reliable conclusions for this species, but some cocoons were found to tolerate temperatures of −1 °C and −5 °C.

L. terrestris, *D. rubidus*, and *D. octaedra* hatched after exposure to −5 °C but their hatchability was significantly lower ($p < 0.05$; chi squared test) than that of the controls. *L. festivus* cocoons are apparently not affected by exposure to −5 °C ($p > 0.05$; chi squared test).

Only *D. octaedra* survived exposure to −10 °C but hatchability was significantly lower ($p < 0.05$; chi squared test) than that of the control.

4. Discussion

The results of this investigation indicate considerable differences in frost tolerance between species. However, most species appear able to withstand a mild degree of frost as all species, with the exception of *E. fetida,* were able to tolerate exposure to −1 °C.

The fact that *A. caliginosa, A. rosea,* and *A. chlorotica* cocoons do not survive temperatures of −5 °C is rather striking as overwintering cocoons are found mostly in the top 10 cm of the soil (GERARD, 1967). The temperature in these soil layers can easily reach well below freezing point during cold spells if no snow cover or dense plant material is present to form an insulating layers. This is often the situation in agricultural soils where large areas of fields may be left bare for the winter. Frost may therefore have a significant negative effect on recruitment in these three species which, together with *A. longa* and *L. terrestris,* are predominant in agricultural soils in Northwestern Europe (GERARD, 1967; ANDERSEN, 1987; BOSTRÖM, 1988). Winter survival of *A. caliginosa, A. rosea* and *A. chlorotica* is probably due to the downward migration of the juvenile and adult worms to warmer soils layers.

However, it cannot be discounted that the relatively high mortality at −5 °C and −10 °C is caused by a lack of acclimatization or a too quick lowering of the temperature in the experiments. In the present study the cocoons were derived from laboratory cultures maintained at 15 °C. In natural situations, however, overwintering cocoons are produced in autumn when the temperature is somewhat lower (EVANS & GUILD, 1948; GERARD, 1967). It is possible that cocoons produced at low temperatures or acclimatized for a longer period would survive frost better than those cocoons used in our experiment. This phenomenon is well known for eggs and adults of many species of arthropods (SØMME, 1982; KIRCHNER, 1986).

SØMME (1982) points out that mortality caused by frost is influenced not only by the temperature but also by the duration of exposure. In our investigation the duration of exposure in the three experiments was different (fig. 1), the −10 °C experiment having the longest exposure time. Thus, the possibility that cocoons can survive shorter time exposures to −10 °C or −5 °C cannot be excluded.

Frost tolerance has almost exclusively been investigated for arthropods, usually by measuring the supercooling point. This has never been examined for earthworm species, but BYZOVA (1974) has proposed that earthworms hibernating in the frozen upper soil layers around Moscow could lower the freezing point of their body fluids by increasing the ionic content. In arthropods, the lowering of freezing point is usually brought about by increasing the concentration of glycerol in the body fluids (SØMME, 1982). This mechanism might well occur in earthworms and also their cocoons.

Our investigation implies that cocoons have a much greater tolerance for frost than have postembryonic individuals (cf. LEE, 1985). This pattern is also found for many arthropod groups (SØMME, 1982). An important factor in this frost tolerance may be that cocoons are free of mineral particles, found in the guts of hatched worms, that might act as nucleators in the formation of ice.

Furthermore, it is possible that changes in frost tolerance occur with the various developmental stages of the embryo; this question needs further investigation.

When the soil in the test tubes freezes, anoxic conditions could arise and increase mortality. Even so oxygen consumption of the cocoons will be very low because of the low temperature (cf. PHILLIPSON & BOLTON, 1976), and in view of the short time span when the soil is frozen we consider oxygen deficiency to have been of minor importance for hatchability in our investigation.

The differences in frost tolerance between the species do not all correspond with their geographical distribution. For example, *A. caliginosa, A. rosea,* and *A. chlorotica* are found as far north as *L. terrestris* (STÖP-BOWITZ, 1969) even though the latter is more frost tolerant. The cocoons of *L. festivus* have a frost tolerance like *L. terrestris* but the distribution of this species is even more southern (STÖP-BOWITZ, 1969).

However, there does seem to be a connection between mode of life and degree of frost tolerance. The endogenic species *A. caliginosa, A. rosea,* and *A. chlorotica* have a lesser tolerance to frost than the two *Lumbricus* species and *A. longa* which all have an anécique mode of life.

Within the epigeic species there is a wide range in frost tolerance. *E. fetida* cocoons are very sensitive, with all degrees of frost being lethal. This species, however, is mostly found in warmer climates and has been introduced by man to northern Europe. Here it is mostly found in compost and dung heaps, protected from frost (STÖP-BOWITZ, 1969).

D. rubidus, which is distributed farther north than *E. fetida*, tolerates frost better. Only *D. octaedra* withstands $-10\,^{\circ}\mathrm{C}$, although hatchability is lowered. This agrees with the fact that *D. octaedra* has the northern most distribution of the species tested (STÖP-BOWITZ, 1969) and is, according to BORNEBUSCH (1930), a pioneer species often found as the only earthworm in extreme habitats. SATCHELL (1980) mentions that it lives as an ephemeral in some tundra areas, apparantly surviving winters as cocoons. This strategy, of course, must be based on cocoons being able to tolerate extreme climatic conditions.

The observed differences in frost tolerance between the species should be considered in relation to their general strategy for winter survival. Some species may be more dependent on cocoon survival during winters than other species.

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Cocoons of 8 species of lumbricid earthworms were exposed to $-1°$, $-5°$, and -10 °C in the laboratory after which hatchability was estimated. Considerable differences in frost tolerance between the species tested were found. *Eisenia fetida* (SAV.) was the most sensitive, not even surviving exposure to -1 °C, whereas *Dendrobaena octaedra* (SAV.) was the most tolerant species, able to survive exposure down to -10 °C.

Frost tolerance does not fully correspond to the geographical distribution of the species investigated, but the results indicate a correlation between ecological type of species and ability to tolerate frost in the cocoon stage.

Key words: Earthworms Lumbricidae, cocoons, frost, frost tolerance, hatchability, ecological type.

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